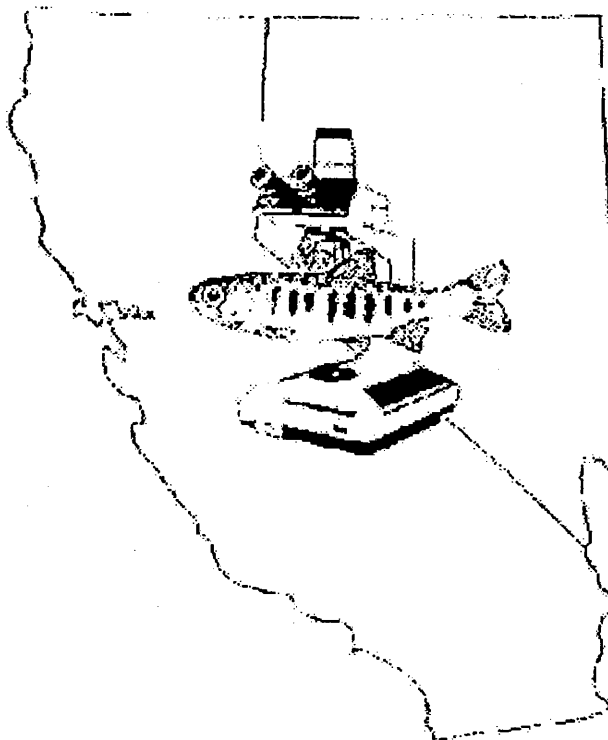# FY2001 Investigational Report :
Juvenile Chinook Health Monitoring in the Trinity River, Klamath River and Estuary. June – August 2001.

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Summary:
Over 650 Chinook salmon (*Oncorhynchus tshawytscha*) juveniles were examined at 5 sites in the Klamath River (R.) basin during the months of June and July 2001. Diagnostic tests were performed on a subset of the examined fish to determine the cause of morbidity. Juvenile Chinook salmon captured in the lower Trinity R. were generally healthy. In contrast, two parasitic infections caused significant sickness and mortality in salmon collected in the Klamath R. and estuary. A myxosporean parasite infection of the kidney induced glomerulonephritis and kidney swelling. The health effect of this kidney infection is unclear. Varying degrees of enteritis were caused by *Ceratomyxa shasta* infection. Ceratomyxosis appears to be the leading cause of mortality and sickness in the Klamath R. and estuary during the summer of 2001.

Elevated water temperature in the Klamath R. and estuary were associated with lower muscle lipid content and gill ATPase activities. Ceratomyxosis resulted in elevated blood neutrophil numbers and lysozyme activity. Plasma protein values were also reduced in affected fish. Future monitoring should concentrate on estuary sampling of marked fish to determine the relative effect of ceratomyxosis on Klamath R. (Iron Gate Hatchery) and Trinity R. (Trinity R. Hatchery) juvenile Chinook salmon. Rapid response to elevated juvenile or adult mortality would be another aspect of a future monitoring program.

The correct citation for this report is:

Introduction:

Anadromous fish populations in the Trinity and Klamath Rivers have declined significantly over the last several decades due to habitat degradation, adverse water quality, and harvest. The hydrology in both rivers was radically altered after Lewiston and Iron Gate dams went into operation. The decline in salmon populations led Congress to enact laws (PL 98-541 and PL 99-552) directing the Secretary of the Interior to take actions necessary to restore fisheries. An intense restoration effort for this valuable aquatic resource has been underway for several decades.

The USFWS California - Nevada Fish Health Center, in cooperation with offices of the California Department of Fish and Game (CDFG), tribal fishery groups and the USFWS, has performed health and physiology evaluations of Chinook salmon juveniles in the Klamath basin since 1991. A number of fish pathogens have been identified from these studies as well as some data on the physiological parameters of Chinook smolts in the basin. Several significant diseases observed to affect Chinook smolts in the basin include infections from *Ceratomyxa shasta, Flavobacterium columnare,* motile aeromonad bacteria, *Nanophyetus salmincola,* and kidney myxosporean parasites (Foott et. al 1999, Williamson and Foott 1998).

The objectives for the project were to:
1) Document the frequency of clinical signs of disease in sick and dead juvenile Chinook salmon captured in the lower portions of the Trinity River, Klamath River, and Klamath estuary.
2) Determine the cause(s) of the observed disease signs from a subset of examined fish and identify trends of disease with water temperature, sample site and date.
3) Compare select physiological parameters of juvenile chinook collected in the Klamath basin with Trinity R. Hatchery juvenile Chinook experimentally reared in elevated water temperatures.

Methods:

Field Collection - Juvenile salmonids (primarily Chinook) were collected from the lower Trinity and Klamath Rivers as well as the Klamath estuary during the period of May 30 to July 30, 2001 (Table 1). Additionally, a single collection occurred from the lower Salmon River on August 3rd and 15 adult Chinook from the Trinity River were examined in early July. This period was chosen to reflect when most Chinook smolts would encounter elevated water temperatures in the

basin. All fish were examined for either external signs of disease (i.e. darken skin coloration, lesions, pale gills, swollen appearance, reduced swimming ability or avoidance behavior). Moribund fish were selected for in-depth examination and a variety of microbiological samples. Approximately 5 – 10 fish of normal appearance were also collected for laboratory examination. Each laboratory sample was assigned a unique accession code (T#) and recorded on the field data sheet to permit for accurate tracking in a database (Appendix 1). Fish were evaluated by a modified organosomatic assay (Goede and Barton 1990, Foott 1990). The organosomatic assay is a method for ordered observation and reporting of the gross morphology of selected organs and size criteria of each individual (Appendix1). Fulton condition factor was calculated from the fork length (KFL = weight (g) / (fork length (mm)^3)x 10^5). Water temperature and dissolved oxygen concentration were measured with a YSI 95 meter at approximate depth of 0.1 m at each sample site. An Onset StowAway ® temperature probe was attached to the Willow Creek Trap (WCT) at a 0.1 m depth on 30May01 and retrieved on 01Aug01. Readings were set for every 2 hours. Other water temperature data was obtained from the Arcata FWO and YSI readings taken at the collection sites (Table 1).

Table 1.
Sample site, date, surface water temperature (°C) ,dissolved oxygen concentration in mg / L (D.O.), and number of juvenile Chinook examined per site (number of other species).

Trinity River

Willow Creek Trap (rkm 34)

Date		Jullian week	°C	D.O.	fish
May	30	22	15.6	9.1	12
	31	22	nd	nd	10
June	1	23	18.3	8.9	7 (1)
	5	23	15.9	9.5	6
	7	23	18.0	9.5	11 (1)
	8	23	19.1	10.1	7
	10	24	17.7	9.2	6 (1)
	12	24	16.0	nd	14
	15	24	17.4	9.9	8 (1)
	19	25	18.6	10.2	8
	20	25	18.0	9.4	7
	21	25	18.9	8.5	9
	26	26	17.4	9.1	6
	27	26	16.9	9.4	8
	28	26	16.6	9.4	6 (1)
July	5	27	23.0	7.9	8
	6	27	23.0	8.3	6
	11	28	22.7	8.4	8
	12	28	21.7	7.6	11
	13	28	22.2	8.3	8
	19	29	20.6	7.8	7
	20	29	21.2	8.7	8
	21	29	21.0	8.5	8
	24	30	22.4	7.7	6 (1)
	26	30	23.0	7.2	6
	27	30	22.7	7.3	7

Total Trinity Fish No. = 213

Salmon River

Date	jullian week	°C	fish
August 3	31	20.5	11 (3)

Klamath River

Pecwan Creek Mouth (rkm 40)

Date		Jullian week	°C	D.O.	fish
June 22		25	22.0	9.0	30 (1)
July 10		28	nd	nd	97

Big Bar Trap (rkm 80)

Date		week	°C	D.O.	fish
June 6		23	18.0	9.5	6
	21	25	21.7	8.1	16
	29	26	nd	nd	11

Total Klamath Fish no. = 160

Lower Klamath Estuary

Date		jullian week	fish
June	11	24	31
		25	26	21
July	2	27	20
	9	28	20
	16	29	20
	23	30	20
	30	31	21

Total Estuary No. = 273

Trinity River Adult Chinook
15 fish examined in July

Laboratory assays - The project's general approach was to diagnose the cause of disease by the simplest method and was based on our knowledge of the pathogens affecting fish in the basin (Foott 1991, Walker and Foott 1992, Foott et. al 1999, Williamson and Foott 1998).

Bacteria and virus – Methanol-fixed imprints of kidney and gill were gram stained and evaluated microscopically for small and filamentous gram-negative rods (presumptive Aeromonid bacteria and *Flavobacterium columnare)*. Each imprint was given a unique tracking number and recorded on the field data sheets. Bacterial media (numbered slant tubes of Brain Heart Infusion and Tryptone Yeast Extract Salt {TYES} agar) were inoculated from the kidney of some moribund fish for bacterial isolation. Samples showing bacterial growth were shipped to the FHC for identification within 3 days of collection. Standard microscopic and biochemical tests (such as API-20E) were used to identify isolated colonies to the genus level. Viral samples were collected when FHC personnel could drive the chilled tissues directly back to the laboratory. Two fish pooled samples of kidney and spleen were assayed for virus on both EPC and CHSE214 cell lines for 15 days at 15°C by standard methods (Thoesen 1994).

Metacercaria and Renibacterium salmoninarum assays - For each field collection, the entire kidney was removed from a minimum of 5 fish and frozen in whirlpak bags uniquely identified with a number code. The specimens were later defrosted, squashed into a thin layer within the bag and examined for metacercaria at 50X magnification with a dissection microscope. A metacercaria score was given to each kidney sample: 0 = none, 1 = 1-20 metacercaria, and 2 = > 20 metacercaria. A direct fluorescent antibody test (DFAT) for *R.salmoninarum* was performed on a digested kidney pellet preparation. The DFAT sample was prepared by adding 1.5 mL of 0.25% trypsin solution (pH adjusted to 8.5 with NaOH) to each sample bag, digesting for 1 hr in a 40°C water bath, and centrifuging the sample at 10,000x g for 20 min. in an Eppendorf microcentrifuge. The supernatant was discarded and 2 smears made from the pellet. The slides were fixed by both heat and then a 5 min. immersion in absolute methanol. They were stained with a polyclonal fluorescent antibody and 50 fields in each duplicate well examined at 100x magnification on an Olympus BHS fluorescence microscope.

Histology – Gill, intestinal tract, pyloric caeca, posterior kidney, and liver tissue was rapidly removed from the fish after death and immediately fixed in Prefer™ fixative (Anatech MI), processed for 5 μm paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined at both low (40X) and high magnification (400X) without knowledge of the sample group. Observational data was entered into a spreadsheet for sample group sorting.

Muscle lipid - Analysis of muscle lipid content was assayed using a modification of the Bligh and Dyer (1959) chloroform methanol (CM) method. A cross section of the caudal peduncle (0.2 – 0.5 g) was dissected, placed into a pre-weighed glass tube with cap and frozen for later analysis.

Plasma lysozyme – The lysozyme activity of plasma (mOD / min.) was determined from 5 μL samples frozen on dry ice, stored at -70°C, and later assayed by the turbidimetric method described by Ellis (1990).

Differential leukocyte counts - Blood smears were air dried, fixed for 5 minutes in absolute methanol, and later stained with Leishman – Giemsa stains (Yasutake and Wales 1983). A differential leukocyte count was performed at 1000x magnification on the first 100 lymphocytes, thrombocytes, neutrophils and monocytes observed on the smear. Because there is poor morphological distinction between the low percentage of rounded thrombocytes (typically spindle-shape) and the more numerous lymphocytes, the combined number of lymphocytes + thrombocytes divided by the number of granulocytes (neutrophils) was used to derive a L{T}: G ratio.

ATPase - Gill Adenosine Triphosphatase activity (ATPase = μmoles ADP / mg protein / hr) was assayed by the method of McCormick and Bern (1989). Briefly, gill lamellae were dissected and frozen in sucrose-EDTA-Imidazole (SEI) buffer on dry ice. The sample was later homogenized, centrifuged and the pellet sonicated prior to the assay. ATPase activity was determined by the decrease over time in optical density (340 nm) as NADH is converted to NAD+. This activity was reported as μmole ADP / mg protein / hr as 1 mole of NAD is produced for each mole of ADP generated in the reaction. Gill Na-K-ATPase activity is correlated with osmoregulatory ability in saltwater and is located in the chloride cells of the lamellae. This enzyme system transports salts from the fish against the concentration gradient to the saltwater.

Results and Discussion

Water quality measurements – Mean daily water temperature ranged from 16.0° to 24.6° C at WCT during the study period of 01 June – 31 July (Fig. 1 and 2). Daily maximum temperatures in excess of 21°C occurred on 6 days in June and throughout the month of July at WCT. The 25.1 °C upper incipient lethal temperature for Chinook salmon was reached on 8 days in July at WCT as a daily maximum (Brett et al. 1982). Dissolved oxygen measurements, at the time of collection (09:00 – 13:00), were above 7.0 mg /L at WCT and considered adequate for salmonids (Table 1). Average daily discharge in the Trinity R. declined from 2556 cfs on 30May to 700 cfs by 30July (CDEC data at Hoopa gauge).

Mean daily water temperature ranged from 18.3° to 25.2 C at the lower Klamath R. Big Bar Trap (**BBT**) during the study period of 01 June – 31 July (Fig. 3). Daily maximum temperatures, in excess of 21°C, occurred on 7 days in June and throughout the month of July at BBT. The 25.1 °C upper incipient lethal temperature for Chinook salmon was reached as a daily maximum on 14 days in July. Dissolved oxygen readings were taken on 2 of the 3 BBT collections and on one Pecwan creek mouth sample. All these June oxygen measurements were above 8.0 ppm and considered adequate for salmonids (Table 1). Water temperature measurements in the lower Klamath estuary ranged from 18 - 22°C and dissolved oxygen concentrations were above 6.0 mg / L for the June and July collections (personal communication Mike Wallace CDFG Natural Stocks Assessment Project, Arcata).

Fish size and condition – The majority of Chinook juveniles sampled for this project were considered to smolts as they were larger than 80 mm in fork length and had silver coloration. The smallest fish were natural Chinook juveniles sampled at WCT prior to the arrival of Trinity R. Hatchery (**TRH**) release smolts (Fig. 4). These fish averaged 64 mm in contrast to smolts sampled past 07June which were > 80 mm in fork length. Chinook juveniles sampled in the estuary were the largest of all collection sites (Fig. 4). Mean condition factor of the sample groups varied between 0.98 and 1.09 (Fig 5).

7

Figure 1.
Daily mean (MDT) and maximum (daily High) water temperature (°C) at Willow
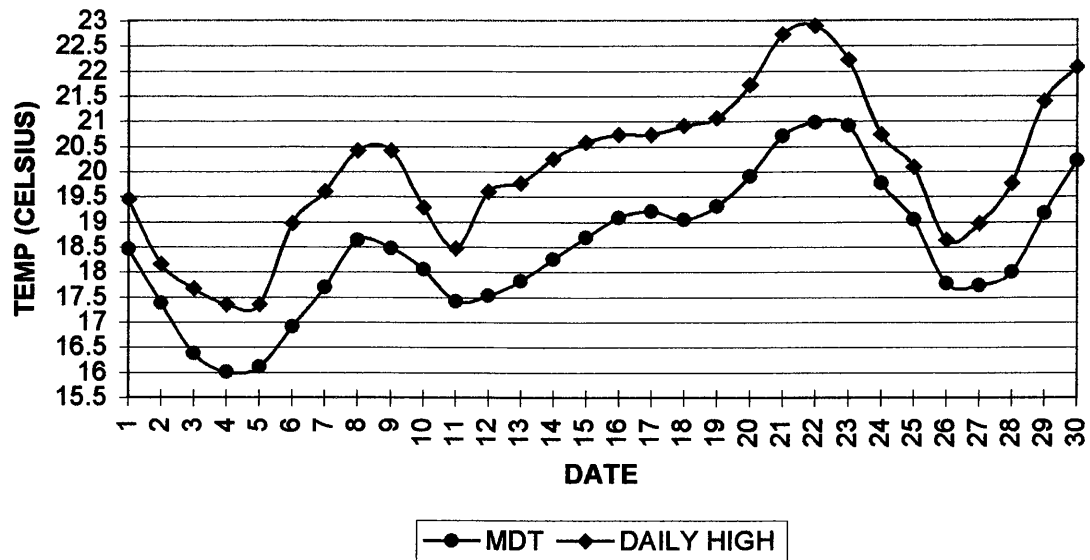Creek Trap in June 2001.

Figure 2. Daily mean (MDT) and maximum (daily high) water temperature
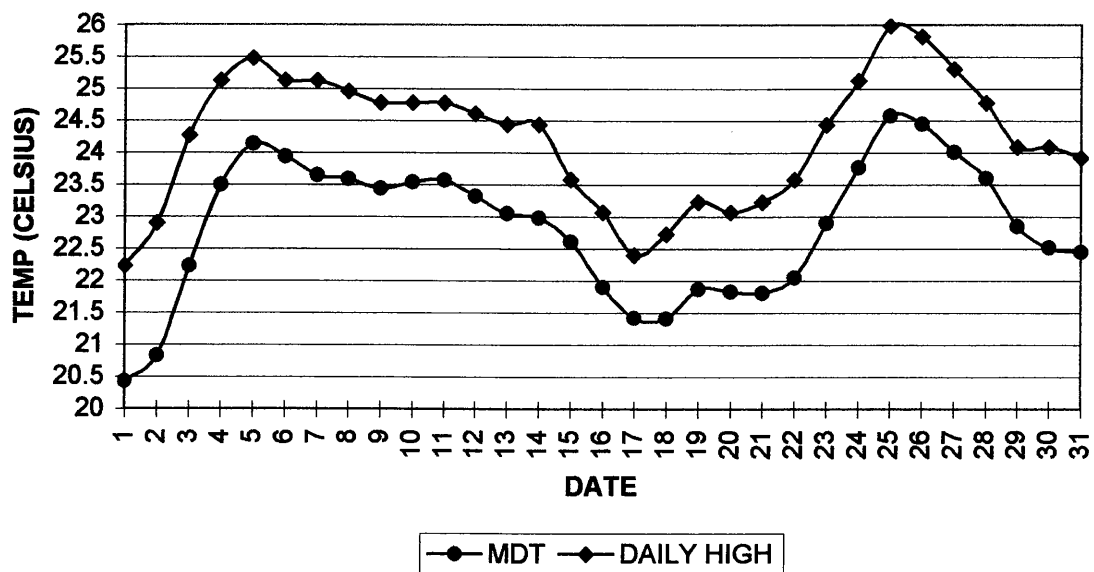(°C) at Willow Creek Trap in July 2001.

Figure 3. Daily mean (MDT) and maximum (max) water temperature (°C) at Big Bar Trap located in the lower Klamath R. in June and July 2001.
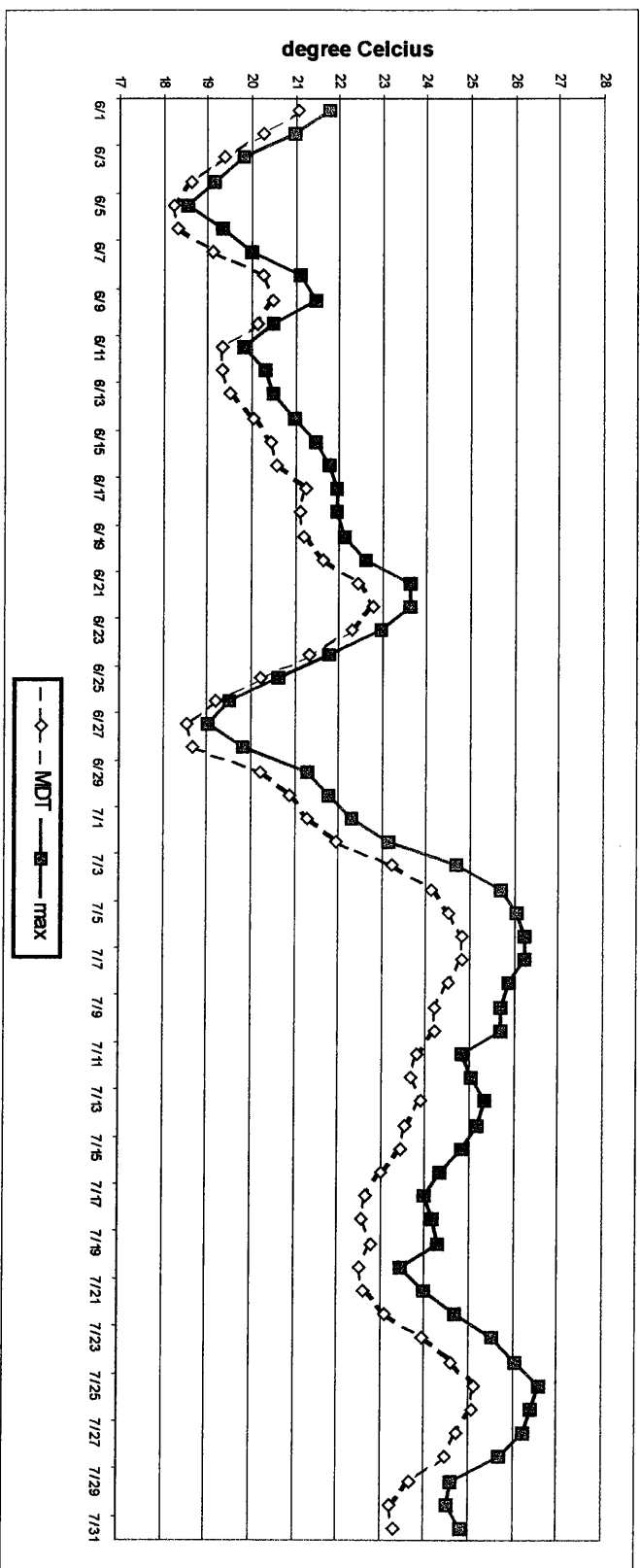
Figure 4. Mean fork length (mm) of juvenile Chinook salmon sampled at Willow Creek (WCT), Big Bar Trap (BBT), Pecwan creek mouth, Klamath estuary (KE), and Salmon R.. Fish captured at WCT prior to hatchery release considered natural origin (nat). Bars indicate standard error of the mean.
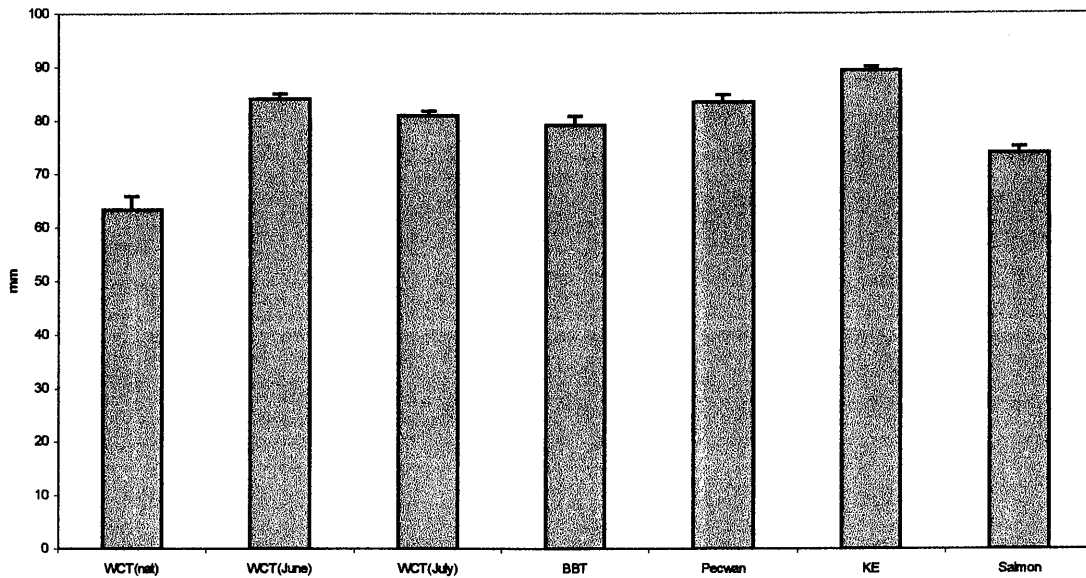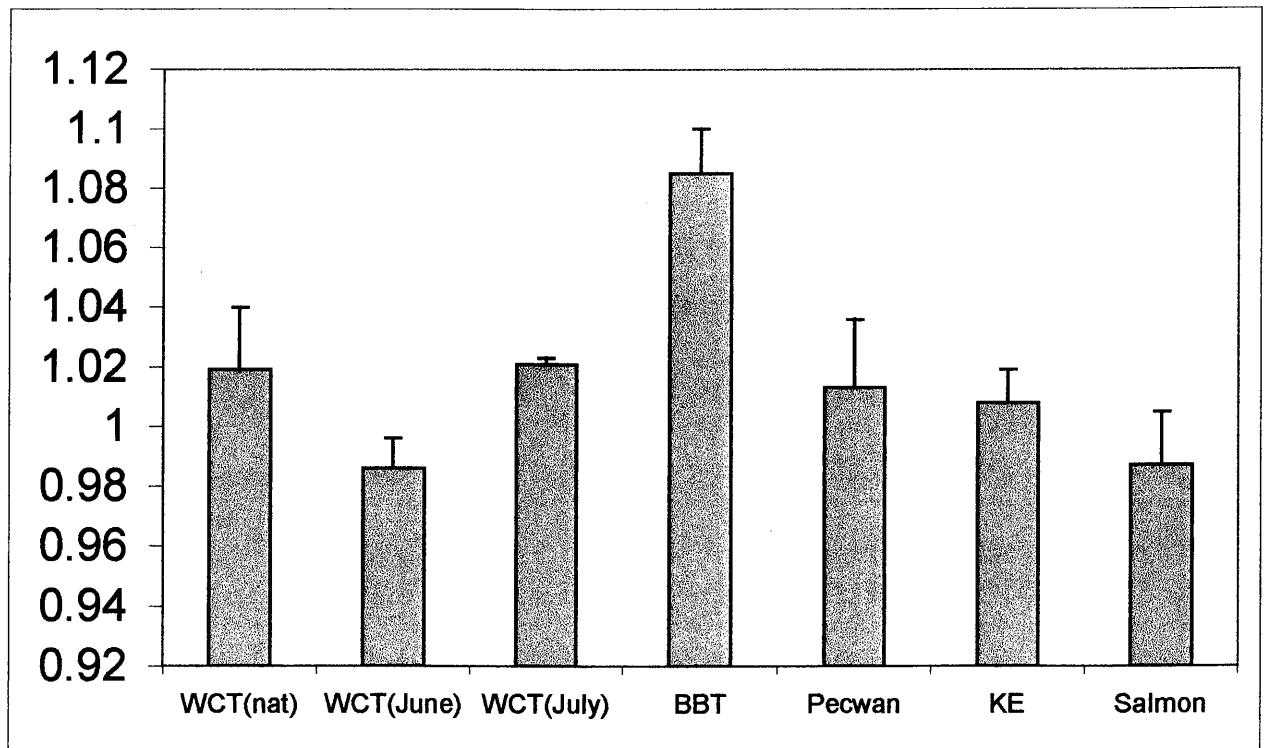

Figure 5. Mean condition factor (Wt/ $FL^3 \times 10^5$ = KFL) of same groups in figure 4. Fish captured at WCT prior to hatchery release considered natural origin (nat). Bars indicate standard error of the mean.

Mortality - Despite evaluated water temperatures in July, the weekly percent mortality remained below 2% at WCT (Fig. 6). Peak mortality occurred during July 10 –15 (Jullian week 28) and followed a period of mean daily water temperatures > 23°C. There were reports of increased adult mortality in the lower Trinity R. during this same 2 week period in early July (personal communication. M. Magneson, Arcata FWO and M. Willis CDFG, Redding). The BBT was operated between 1 and 6 days per week during the study period. This schedule limits analysis of mortality for this site, however, there was considerably higher mortality than at WCT (Fig. 7). A peak weekly mortality of 60 % (1033 mortalities / 1730 total juvenile salmonids) occurred during a 4 day collection period between 29May – 03June. Mean daily water temperature was $\leq$ 21 °C at this time. Moribund Chinook juveniles were observed near Pecwan creek mouth on both the 22June and 10July sample dates. Ceratomyxosis was the predominant health problem at these 2 Klamath R. sites.

No estimate of mortality was determined in the lower estuary. Approximately 2 – 12 % of the 0+ Chinook collected in beach seine catch was sampled in the lower estuary during June and July. The highest catch occurred in the thermal refugia zone associated with the mouth of Hunter creek (personal communication Mike Wallace CDFG Natural Stocks Assessment project Arcata). Close correlation between trap mortality and water temperature at a given site may not be possible as we do not have a thermal history of fish prior to their capture. The effect of upstream thermal refugia or zones of elevated water temperature could be a confounding factor.

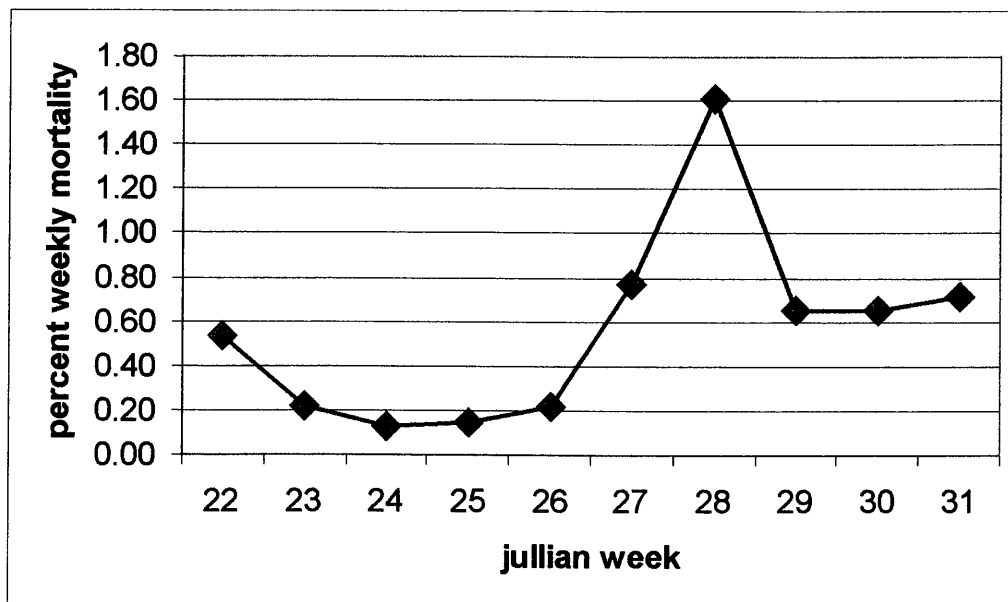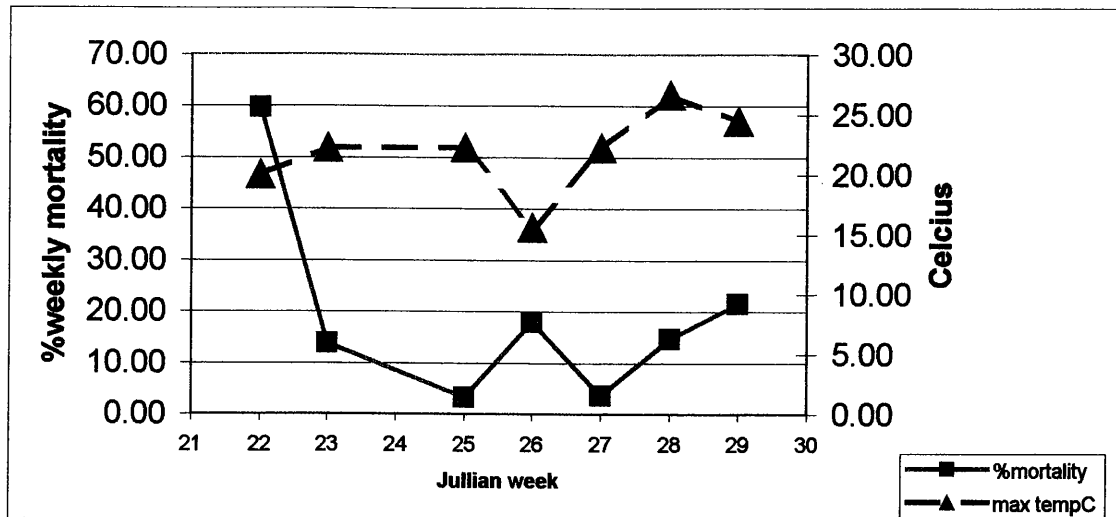Figure 6. Percent weekly mortality at WCT .

Figure 7.
Percent weekly mortality at BBT. Trap operated between 1 and 6 days per week.

Adult samples – In early July, reports of adult Chinook mortality in the lower Trinity R. were received from biologists with CDFG, Hoopa Fisheries, and USFWS. This period corresponded to rapidly raising water temperatures in both the lower Klamath and Trinity Rivers (Figs 1 - 3). The FHC received samples from 8 adult Chinook and examination data from 15 fish. Details are listed below:

a.   10July - Two moribund fish collected below Junction city by CDFG drift boat crew and held on ice for 24 hrs prior to arrival at FHC.
- viral sample was negative for cytopathic effects on 2 cell lines
- histological examination of intestine and kidney sections were limited by post-mortem necrosis, however, one intestine sample showed clinical signs of Ceratomyxosis (inflammation and epithelial erosion).

b.   10July - One moribund fish sampled for histological samples by USFWS crew. The external and internal features of the fish were rated as normal by the crew. Unfortunately, the histology samples were destroyed in a processing mishap.

c.   14July - Seven fish caught in the Hoopa gill net fishery near Tish Tang were examined for clinical signs of infection. Two of the 7 fish had necrotic gills indicative of Columnaris. No bacteria were observed in a gram-stained kidney imprint from one apparently healthy fish.

d.	20July - Five fish caught in the Hoopa gill net fishery near Tish Tang were examined for clinical signs of infection. Four of the five fish were reported to have pale gills, however, no other clinical signs of disease were noted in them. The 5 BHIA cultures collected from these fish contained *Bacillus* sp.. These isolates were considered contaminants and not representative of systemic infections.

The limited laboratory results and field observations do not allow for conclusive diagnosis of the cause(s) of the adult mortality. Diagnosis will require the capture and rapid necropsy of sick fish. This is a difficult task in a large river system. One possible cause for the mortality episode may be a combination of elevated water temperatures in the lower rivers and infection by *C. shasta* and *F. columnare*.

	Clinical signs of disease – Similar to the low trap mortality, few Chinook examined at WCT showed any external or internal abnormalities such as pale gill, hemorrhagic intestine, or swollen kidney. Columnaris gill lesions were seen in 2.8 % of the smolts examined over the entire study period. Minor hemorrhagic spots (petechia) were recorded on the skin of 4.7 % of the 213 smolts examined at WCT. It is unclear what role trauma, due to trapping and handling, played in this skin condition as bacterial infections were rarely detected at this site.

We observed pale gills (anemia) and varying degrees of intestinal hemorrhaging in Chinook collected from both the Klamath R. and estuary. Both clinical signs tended to occur in the same fish and were due to the enteritis caused by *C. shasta* infection. Histological examination of tissues from affected fish confirmed this diagnosis. The incidence of hemorrhagic intestine ranged from 10 – 65 % in the estuary collections during the jullian weeks of 27 – 31 (Fig. 8). This period is highlighted as only one individual (R. Engle) was rating the samples. No obvious temporal trend was observed in the data. The highest incidence of Ceratomyxosis signs occurred in the 16July sample (jullian week 29).   The incidence of pale gills and hemorrhagic intestine in moribund Klamath R. fish ranged from 41 – 64 % and 57 – 77 %, respectively.

Swollen kidneys were seen in 10 – 55 % of the estuary fish (Fig. 8) and 45 – 74% of the Klamath R. sample groups. Histological examination of fish with swollen kidney clearly link it with the glomerulonephritis induced by the myxosporean parasite infecting the kidney. We cannot directly compare the incidence of disease from the estuary and Klamath R. due to different sampling biases. Sick fish were selectively sampled in the Klamath R. while no such selection occurred with the estuary samples. Another confounding factor is the subjective nature of assigning clinical signs by the 4 individual collectors. Given these limitations, it is still apparent that large numbers of Chinook smolts migrating through the Klamath R. were severely impaired due to parasitic infections.

Figure 8.
Prevalence of clinical signs in Chinook sampled in the estuary. Signs included pale gills, hemorrhagic intestine (hem. intes.), and swollen kidney (swol.kd.).
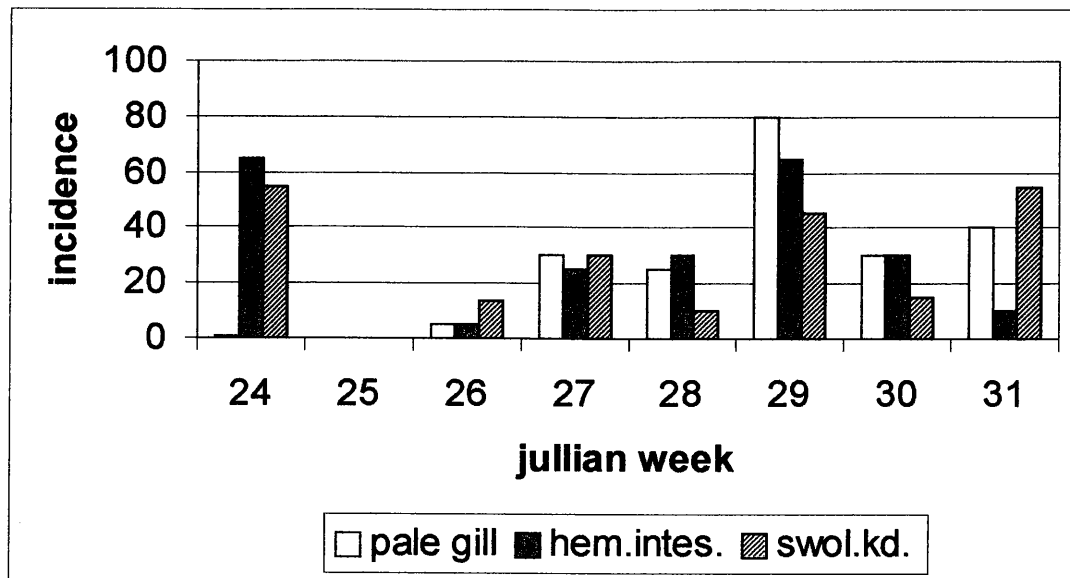
Histological examination results – Parasitic infection and abnormalities were evaluated in tissue sections from 117 fish collected at WCT, BBT, Pecwan creek mouth, estuary, and Salmon R. (Table 2). Metacercaria were observed in both the gill and kidney sections of fish from all sample sites including the Salmon R. Gill infections were much more prevalent than in the kidney. The encysted metacercaria were often associated with hyperplastic branchial cartilage that distorted the normal gill architecture. The number of parasites per gill filament did not appear to be high enough to significantly impair gill function. The metacercaria were presumptively identified as the trematode *Nanophyetus salmincola.* Glochidia, larval mussel presumptively identified as the genus *Margaritifera,* were also seen in 17 % of the WCT fish.
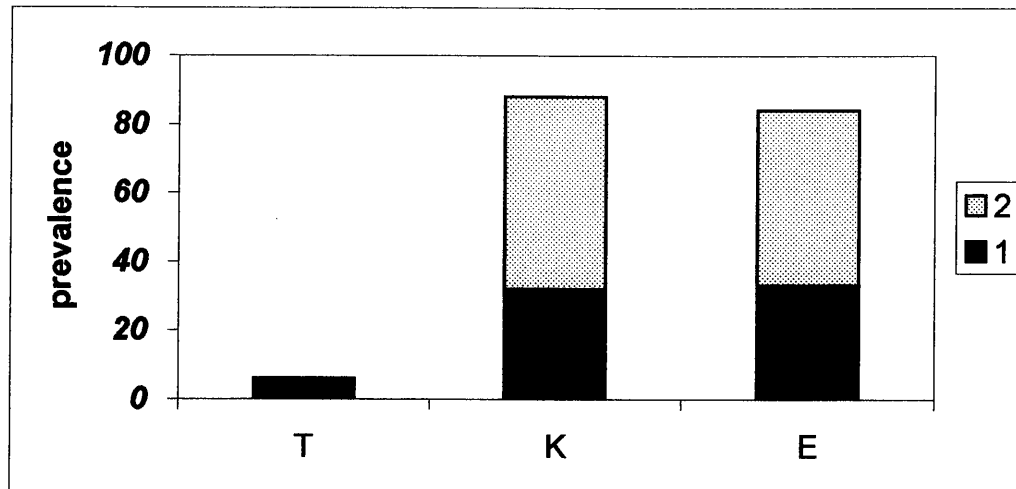
Table 2.

Histological examination results of fish collected at Willow Creek Trap (WCT), Big Bar Trap (BBT), mouth of Pecwan Creek (Pecwan), lower Klamath Estuary (Estuary) and Salmon R. (Salmon). Data given as number of specimens positive / total specimens (%) for a given severity score of infection by *Ceratomyxosis shasta* (*Cshasta*), Kidney myxosporean parasite (Kd Myxosp), trematode metacercaria in the kidney (Kd Metac.), inflammation of adipose tissue adjacent to the pyloric caeca (Adipose Infl.), metacercaria and glochida (Gloc.) in the gill. Severity score of 1 indicates presence of parasite or mild inflammation without severe lesion. A score of 2 is associated with a severe lesion.

| | *C.shasta* | | Kd Myxosp | | Kd Metac. | Adipose Infl. | | Gill | |
	1	2	1	2		1	2	Metac.	Gloc.
WCT	0 / 38 (0%)	0 / 38 (0%)	2 / 31 (6%)	0 / 31 (0%)	6 / 31 (19%)	1 / 24 (4%)	1 / 24 (4%)	15 / 24 (63%)	4 / 24 (17%)
BBT	1 / 14 (7%)	5 / 14 (36%)	5 / 12 (42%)	5 / 12 (42%)	1 / 12 (8%)	3 / 8 (38%)	1 / 8 (13%)	7 / 10 (70%)	0 / 10 (0%)
Pecwan	0 / 20 (0%)	11 / 20 (55%)	3 / 13 (23%)	9 / 13 (69%)	0 / 13 (0%)	4 / 12 (33%)	4 / 12 (33%)	11 / 16 (69%)	0 / 16 (0%)
Estuary	1 / 42 (2%)	11 / 42 (26%)	14 / 43 (33%)	22 / 43 (51%)	5 / 43 (12%)	2 / 22 (9%)	16 / 22 (73%)	18 / 24 (75%)	0 / 24 (0%)
Salmon	0 / 3 (0%)	0 / 3 (0%)	0 / 3 (0%)	0 / 3 (0%)	0 / 3 (0%)	0 / 3 (0%)	0 / 3 (0%)	3 / 3 (100%)	0 / 3 (0%)

The pre-sporogonic stage of a myxosporean parasite was observed in the glomeruli and within the renal tubule lumens of Chinook kidney sections from the estuary, Klamath and Trinity Rivers (Table 2). The incidence and severity of infection was similar in the estuary and Klamath R. samples and considerably greater than that seen at WCT (Fig. 9). The infections produced varying degrees of interstital hyperplasia and glomerulonephritis that, when severe, resulted in swollen kidneys. It is likely that kidney function would be impaired due to the glomerulonephritis. In late July, a number of estuary samples only contained the parasite in the renal tubules. The glomeruli appeared normal in these fish. This observation suggests that fish may recover from the infection given enough time. It is unclear how many myxosporean species are present in the kidney samples. No definitive genus identification was possible due to the lack of identifying spores in all but one sample. One WCT specimen, collected on 12July, contained spores of *Chloromyxum sp* in large glomerular cysts. Similar cysts were not seen in the other positive kidney sections. Several other possible candidates include *Myxidium minteri, Parvicapsula sp.* , and *Sphaerospora sp.* (Yasutake and Wood 1957, Kent et al. 1994). Meyer and McPherson (1985) describe a similar myxosporean in adult Rogue R. Chinook suffering from chronic glomerulonephritis. These authors did not observe spore stages and were also unable to identify the parasite.

Figure 9.

Incidence of kidney myxosporean infection in Chinook juveniles sampled in the Trinity R. (T), Klamath R. (K), and estuary (E). Severity score of 1 indicates presence of parasite or mild inflammation without severe lesion. A score of 2 is associated with a severe lesion.

Varying degrees of inflammation were observed in the adipose and acinar cells covering the pyloric caeca (peripancreatic region) of Chinook from the estuary, Klamath and Trinity Rivers (Table 2). The incidence and severity of infection was markedly higher in the estuary and Klamath R. samples (Fig. 10). Severity tended to increase with time in the estuary samples and may be related to the duration of lipid mobilization by the fish in warm waters. Many of the July samples showed diffuse granulomas (scar tissue) in this region reflective of chronic inflammation. Several such sections also contained lipofuscin pigment. Lipofuscin is an insoluble residue of oxidized phospholipids and is an indicator of oxidative stress. We do not believed that this condition is related to Ceratomyxosis as it can be observed in fish held in waters free of this parasite such as at WCT and in laboratory experiments. The enzymatic mobilization of lipids from the adipose cells could result in an associated release of arachidonic acid from the cell membrane phospholipids. Arachidonic acid can be converted to a number of potent chemotactic factors for phagocytes such as leukotrienes and salmonids are reported to generate such agents (Secombes 1996). Once phagocytes are recruited to the region they would release other chemotactic factors (cytokines) that would further enhance the inflammatory response. The observation of leukocytes migrating from blood vessels in the peripancreatic region of smolts collected early in the study lends some evidence to an initial release of a chemotactic factor(s). We have not located any references to either steatitis or pancreatis in juvenile Pacific salmon to help interpret whether these are" abnormal" lesions for smolts.

Figure 10.
Incidence of peripancreatic adipose tissue inflammation in Chinook juveniles sampled in the Trinity R. (T), Klamath R. (K), and estuary (E). Severity score of 1 indicates early mild stages (perivascular) while a 2 rating describes diffuse inflammation and / or granulomatous response.
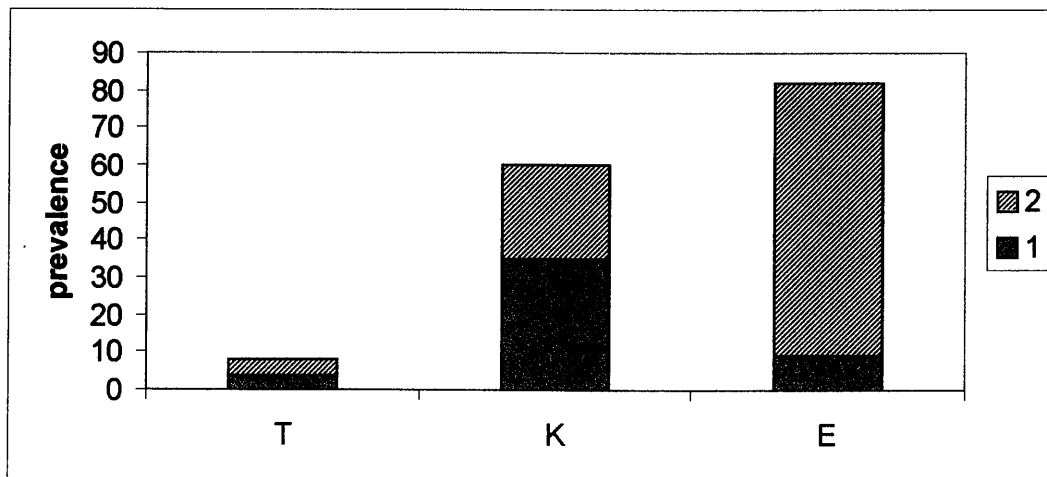
Ceratomyxosis appeared to be a significant health problem and mortality factor for Klamath R. and estuary Chinook smolts. *Ceratomyxa shasta* infection was observed in 50% of the smolts sampled in the Klamath R. (BBT and Pecwan) and 28 % of estuary smolts (Fig. 11). One fish sampled at Pecwan with clinical Ceratomyxosis was a 40 mm coho salmon. This diagnosis was confirmed by histology. In past years, we have seen a similar pattern of lower incidence of infection in the estuary compared with the Klamath R and attribute it to substantial mortality of infected fish prior to reaching the estuary (Foott et al. 1999, Williamson & Foott 1998). The majority of these infections were judged to be severe (#2 rating) due to extensive tissue damage and inflammation. The focal nature of Ceratomyxosis was demonstrated in many specimens where the parasite was seen in the liver or free in the peritoneum, and not the sectioned portion of intestinal tract. *Ceratomyxa shasta* infection was not observed in specimens from the Trinity or Salmon Rivers. Whether Ceratomyxosis affects Trinity R. smolts could not be evaluated in the data set as only 2 adipose-fin marked fish were sampled for histology in the estuary. Neither *C. shasta* nor the myxosporean kidney parasite was detected in either of these TRH marked fish (tag identity personal Communication. M. Wallace CDFG Arcata). Direct mortality due to the disease is not the only outcome of Ceratomyxosis. The hemorrhagic anemia associated with advanced Ceratomyxosis would weaken the fish and increase its chances of predation. Mesa (1998) reports how Chinook juveniles with clinical BKD infections suffer a higher rate of predation than non-infected cohorts. Entry into the ocean would not aid the infected fish as Ceratomyxosis is reported to continue after entry of infected salmonids into saltwater (Kent et al. 1994).

Figure 11.
Incidence of *Ceratomyxa shasta* infection in Chinook juveniles sampled in the Trinity R. (T), Klamath R. (K), and estuary (E). Severity score of 1 indicates presence of parasite or mild inflammation without severe lesion. A score of 2 is associated with a severe lesion.
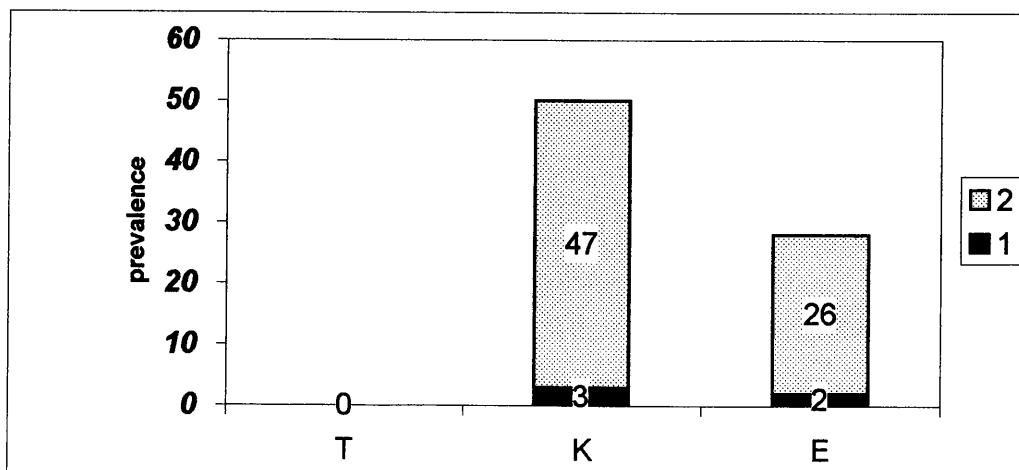
Most salmonids are susceptible to varying degrees of Ceratomyxosis (Johnson 1980). Many workers describe steelhead and salmon stocks from enzootic waters being generally more resistant to Ceratomyxosis than naive stocks (Zinn et al. 1977, Ching & Munday 1984). The genetic component to this resistance has been demonstrated in a reciprocal crossing experiment with F1 trout from resistant and susceptible stocks (Ibarra et al. 1992). This innate resistance could be overwhelmed by long -term exposure to infectious water or intraperitoneal injection of the parasite (Ibarra et al. 1991). It is unclear what defensive mechanisms inhibit the multiplication and dissemination of the parasite in resistant stocks. Fryer (1987) reports that naturally infected rainbow trout did not produce specific antibody to the parasite and that exclusion from the lower intestinal epithelium may be an important resistance mechanism. Hendrickson et al. (1989) reports that the infective stage of *C.shasta* is found in the Sacramento, San Joaquin, Pit, and Klamath R. systems. In the Klamath basin, juvenile salmonid infection with *C.shasta* occurs in the Klamath R. but not in the Trinity R. (FHC monitoring data 1991 - 1998, Hendrickson et al. 1989). The parasite has a limited geographic range in spite of the occurrence of infected adult salmonids migrating into non-infective waters. The lifecycle of *C. shasta* apparently involves an alternate host found in specific waters. Bartholomew et al. (1997) reported that an actinosporean released from the polychaete *Manayunkia speciosa* could infect trout with *C. shasta* . Natural infections are reported to occur when water temperatures are above 7 °C and in the lower Klamath R. began in April and ceased after December (Hendrickson et al. 1989). Iron Gate hatchery fall-run Chinook and Steelhead juveniles have been shown to be resistant to Ceratomyxosis when challenged to the infectious stage in Pit R. water at $\leq$ 16°C or released in November (M. Willis, August 1, 1996 memo, Appendix 2, Foott et al. 1999). Increased water temperatures have been shown to inversely effect the survival of salmonids infected with *C. shasta* (Ching & Munday 1984, Udey et al. 1975). The mean time to death dropped from 45 - 51 days at 5 - 6 °C to only 27 days at 17 °C (Ching & Munday 1984). Coho juveniles held at 23.3 °C had a geometric mean time to death of 12.5 days post - exposure that increased to 38.5 days at 17.8 °C (Udey et al. 1975). The percent mortality of exposed coho held at 15 °C or below was $\leq$ 21 % but jumped to 53 % at 17.8 °C and 84 % at 20.5 °C. At temperatures below 6.7 °C, exposed Coho were completely resistant to Ceratomyxosis (no deaths or spore detected). This inability to induce disease or sustained infection (spore production) in Coho was probably due to host defense mechanisms and not temperature inhibition of parasite.

Metacercaria – The incidence of metacercaria infection detected in kidney squash preparations was as follows: 23% Trinity R., 23% estuary, 47% Klamath R., and 20 % in the 03August Salmon R. collection. Approximately half of the positive samples were rated at the higher number 2 severity classification (>20 metacercaria). It is unlikely that these infections were a significant health threat to the fish (Foott et al. 1997, Foott 1996). Both the incidence and severity of

metacercaria infection was lower than seen in the early 1990's. In 1992, we observed infections in > 90 % of the Trinity R. Chinook smolts at a maximum severity of 33,000 metacercaria per gram of kidney (Foott et al. 1994). Metacercaria were also seen in 63 – 100% of the histological sections of gill from the same sites (Table 2). The metacercaria were presumptively identified as the trematode *Nanophyetus salmincola.*

Nanophyetus salmincola is found in salmonids and other associated freshwater fish (cottids, cyprinidae, lamprey) throughout the Pacific Northwest (Millemann and Knapp 1970). Its range is limited to waters that are habitat for its intermediate *Juga* sp. snail host. The pathogenicity of the trematode to fish is reported to vary considerably and appears to be a function of accumulation rate, fish size, species and stock susceptibility, infection site(s), and absolute number of metacercaria (Necomb et al. 1991, Wood and Yasutake 1956, Baldwin et al. 1967, Milleman and Knapp 1970). The life cycle of *N. salmincola* starts with the release of eggs from the adult trematode into the intestine of its final host, a piscivore such as an otter, bear, raccoon, heron, or merganser and then pass out into the water with host's feces. A ciliated miracidium stage hatches from the egg, penetrates a snail host (*Juga sp.*), asexually multiples, and eventually produces a xiphidiocercaria (cercaria with oral sucker stylus which is motile by use of its tail). The cercaria will seek out a fish host and rapidly burrow into the skin, lose its tail, and migrate through the circulatory system to various tissues such as the gill, heart, liver, muscle, optic nerve, and kidney. The parasite (now referred to as metacercaria) tends to concentrate in the posterior kidney due to the migration path through the renal portal system (Milleman and Knapp 1970). The metacercaria will remain with the salmonid fish throughout its saltwater phase and completes its lifecycle after the fish is eaten by a final host.

   Bacterial and Viral infections – R. salmoninarum was not detected by DFAT in 207 kidney samples collected at the 3 sites (Table 3). Bacterial kidney disease does not appear to be a health threat to the Klamath basin's smolt population (Foott 1996). No bacteria were observed in 204 gram-stained imprints of kidney or spleen tissue. These samples were collected from both apparently normal fish and a some fish showing petechial hemorrhaging of the skin. Kidney imprints were deemed a poor sample for bacterial detection as a large portion of them did not stain adequately and extensive microscope time was required for examination. We suspect that delayed fixation of many imprints impaired their staining quality. Motile, gram-negative , cytochrome oxidase positive bacteria (presumptive *Aeromonas hydrophilia* or *Pseudomonas sp.*) were isolated from 36 % of the BHI agar cultures taken from moribund fish (Table 3). Gill and body lesions suggestive of Columnaris disease were seen in only a few fish from the sample sites (approximately 10 out of 660 juveniles). *Flavobacterium columnare,* the causative agent for Columnaris disease, was observed in gram stained imprints of affected gills and isolated on TYES agar (Table 3). Columnaris disease did not appear to be an significant health problem for the 2001 spring migrants. Both motile aeromonad bacteria and *F. columnare*

are common inhabitants of freshwater (Thune et al. 1993). No virus was isolated from fish collected at the 3 sites (Table 3).

Table 3. Incidence of infection of *Renibacterium salmoninarum* by direct fluorescent antibody test of kidney (Rsal DFAT), aeromonid / pseudomonid bacteria cultured from the kidney (A/P), *Flavobacterium columnare* cultured on TYES agar (Fc-TYE) or observed in gram stain imprints of necrotic gill (Fc-Gill), and virus from kidney-spleen samples. Data presented as number positive / total sample (%).

	Klamath R.	Estuary	Trinity R.	Basin total
Rsal DFAT	0 / 47 (0)	0 / 30 (0)	0 / 130 (0)	0 / 207 (0)
A/P	3 / 13 (23%)	10 / 25 (40%)	3 / 7 (43%)	16 / 45 (36%)
Fc-TYE	1 / 2 (50%)	0 / 6 (0)	1 / 22 (5%)	2 / 30 (7%)
Fc-Gill	0 /1 (0)	ND	6 / 8 (75%)	6 / 9 (67%)
Viral	0 / 30 (0)	0 /14 (0)	0 / 14 (0)	0 / 58 (0)

Physiological data.

Physiological measurements, reported below, were performed on blood and tissue samples from Chinook juveniles collected by the lead author at the following locations:

31May	WCT	Trinity R. natural smolt (prior to TRH release)
12June	WCT	mix of TRH and natural smolts
22June	Pecwan	moribund smolts in the lower Klamath R.
11June	estuary	mix of hatchery and natural fish

These various measurements are compared to TRH Chinook experimentally reared for 14 days at a MDT of 23.7°C (unpublished 2001 CA-NV FHC study, report pending).

Lipid - Caudal muscle percent lipid was lower in Chinook smolts collected from the lower Klamath R. (Pecwan 6/22) and estuary than those fish collected at WCT or directly from TRH prior to any temperature challenges (Table 4).

Table 4.   Mean (± SEM) percent lipid of caudal muscle.

Estuary 6/11	Pecwan 6/22	WCT 6/12	TRH 6/25
1.78 (± 0.19)	1.79 (± 0.24)	2.44 (± 0.15)	2.83 (± 0.10)

While all three in-river groups must be considered a mix of both natural and hatchery fish, it is likely that the 12June WCT sample was composed primarily of TRH fish released approximately a week earlier. These fish had similar lipid content as their cohorts held back at TRH. Lower lipid values seen at Pecwan and the estuary indicate the higher energy demands of migration in warm waters.

Gill ATPase activities - Elevated temperature appears to have a negative effect on ATPase activities. Despite their similar size, the smolts at Pecwan and the estuary had lower ATPase values than fish collected from the cooler Trinity R. (WCT). A similar ATPase inhibition was observed in TRH Chinook exposed to a MDT of 23.7°C in a 14 day laboratory study (data from unpublished FHC 2001 study listed in Table 5). The Klamath R. fish captured at Pecwan and the 14 day experimental fish both showed ATPase activities reflective of impaired smolt development.

Table 5.

Mean ($\pm$ SEM) gill ATPase activity (ATPase = μmoles ADP / mg protein / hr) and
fork length of Chinook smolts captured at Willow creek trap (WCT), estuary,
Pecwan creek mouth, and TRH fish experimentally challenged for 14 days at a
mean daily temperature of 23.7°C. Collection group identified as either of natural
or presumed mixed natural and hatchery origin (Mixed). Water temperature at
each collection site reported.

| Site / Date / Group	ATPase Activity	Fork length
WCT 31May Natural N = 5 15.6°C	9.49 ± 1.48	78 ± 5
WCT 12June Mixed N = 11 16.0°C	12.17 ± 1.09	103 ± 4
Estuary 22June Mixed N = 10 17°C (surface)	7.18 ± 0.77	102 ± 3
Pecwan 22June Mixed N = 9 22.0° C	3.50 ± 0.51	90 ± 3
TRH start of experiment N = 10 , 6 days at 17 - 19°C	7.91 ± 0.67	94 ± 1
TRH 14 days of exposure N = 9 MDT 23.7 °C	4. 83 ± 0.32	93 ± 1

Blood leukocyte counts - Differential leukocyte counts demonstrated
elevated neutrophil numbers in many blood smears from fish infected by
myxosporean parasites but was not strictly associated with elevated water
temperatures (Table 6). Neutrophils are the dominant granulocyte cell found in
the blood of salmonid fishes and function as phagocytes (Yasutake and Wales
1983). Higher granulocyte counts will shift the ratio of Lymphocytes to
Granulocytes (L:G) and can indicate infection, tissue damage, or seasonal blood
cell changes (Modra et al 1998). The fish's response to parasite infection is
reflected in the ow L:G ratios seen in Pecwan and estuary smolts (Fig.12). All fish
from the 22June Pecwan and 11June estuary blood smear groups showed
clinical signs of Ceratomyxosis as well as infection by the kidney myxosporean.
Both parasites were also observed in histological sections from these 2 groups.
This trend was not absolute as several fish with low neutrophil counts were
severely infected with both parasites. High water temperature does not appear
to induce neutrophilia. The estuary sample occurred when surface water
temperature was a relatively low 17°C. Also, Chinook smolts experimentally
reared at a MDT of 23.7° C for 14 days (14dTRH) maintained a LT: G ratio
similar to the natural Chinook sampled at WCT on 31May.

Table 6.

Mean percent (± SEM) lymphocyte, neutrophil, and Lymphocyte + Thrombocyte :
Granulocyte (LT:G) ratio observed in blood smears from Chinook smolts
captured at Willow creek trap (WCT), estuary, mouth of Pecwan creek, and TRH
fish experimentally challenged for 14 days at a mean daily temperature of
23.7°C. Collection group identified as either of natural or presumed mixed
natural and hatchery origin (Mixed). Water temperature at each collection site
reported.

Site / Date / Group	lymphocyte	neutrophil	LT:G
WCT 31May Natural N = 8 15.6°C	85 ± 4	2 ± 1	67 ± 21
WCT 12June Mixed N = 12 16.0°C	83 ± 2	3 ± 1	47 ± 8
Estuary 22June Mixed N = 12 17°C (surface)	88 ± 3	5 ± 1	37 ± 10
Pecwan 22June Mixed N = 7 22.0° C	85 ± 2	9 ± 2	14 ± 4
TRH 14 days of exposure N = 12 MDT 23.7 °C	88 ± 2	2 ± 1	61 ± 11

Figure 12   Lymphocyte (+thrombocyte) to Granulocyte ratios of Chinook
juveniles sampled at Willow Creek Trap (WC), estuary (KE), Klamath R near the
mouth of Pecwan creek (KR) and TRH fish experimentally challenged for 14
days. Dates precede location code.
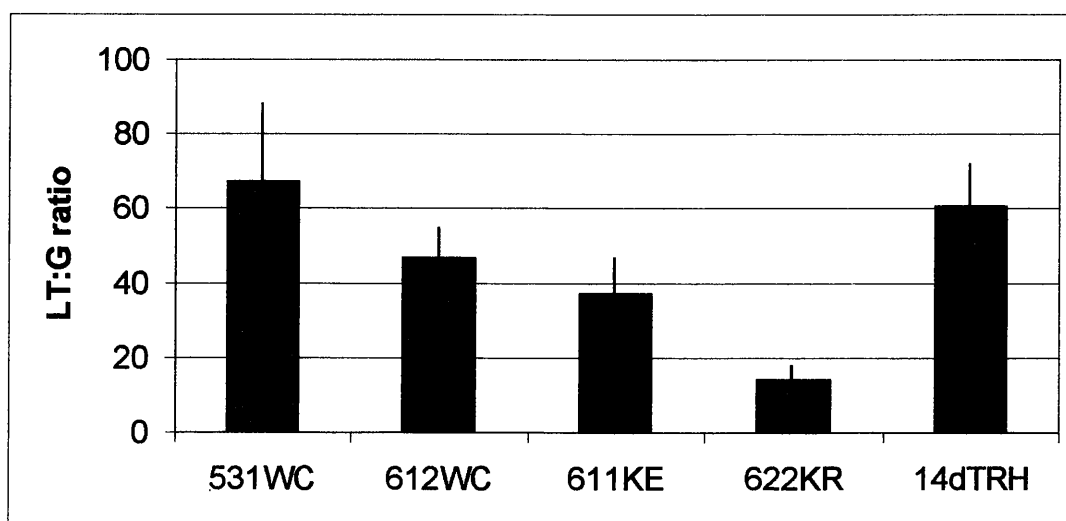
Plasma lysozyme – The effects of myxosporean infection were also apparent in the markedly higher plasma lysozyme activity of smolts from the estuary and Klamath R. (Fig. 13). Trinity R. smolts had 4 – 8X lower activities than the 11June estuary and 22June Pecwan collection groups. Lysozyme is produced by phagocytes and acts to degrade the cell wall of bacteria (Lawrence 1992). Its activity in a given volume of plasma is an indicator of phagocyte number and activity (Ellis 1990). Chinook smolts experimentally reared at a MDT of 23.7° C for 14 days had lysozyme activities (mean 23.8 mOD/min) that were intermediate of the WCT and infected Chinook (Fig. 13).

Fig. 13   Plasma lysozyme activity (mOD / min.) of 5 µL plasma samples from Chinook smolts collected at Willow creek trap (WCT), estuary, Pecwan creek mouth, and TRH fish experimentally challenged for 14 days at a mean daily temperature of 23.7°C.
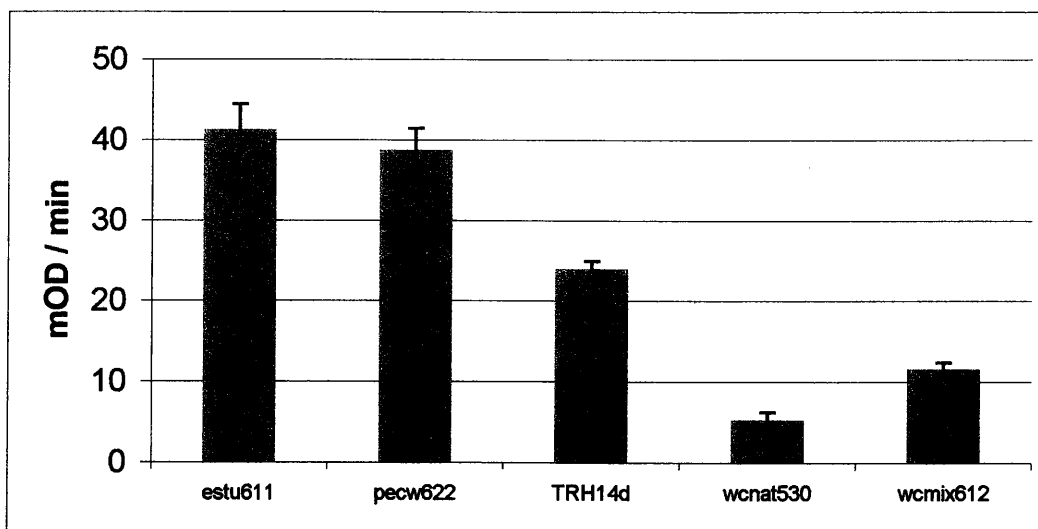
Plasma protein- Protein values were quite low (hypoproteinemia) in estuary and Pecwan smolts with severe enteritis due to Ceratomyxosis (Table 7). The degree of enteritis was rated for each fish in these 2 groups by either clinical signs (hemorrhagic intestine) or histology results. The loss of blood from the intestine was the most likely cause of the observed hypoproteinemia. Chinook smolts experimentally reared at a MDT of 23.7° C for 14 days had plasma protein concentrations that ranged from 2.7 – 4.2 mg / dL. These values were higher than observed in Trinity R. smolts sampled at WCT and may reflect daily feeding of a high protein diet.

Table 7.
Mean plasma protein concentrations (mg / dL) in Chinook smolts captured at Willow creek trap (WCT), estuary, mouth of Pecwan creek, and TRH fish experimentally challenged for 14 days at a mean daily temperature of 23.7°C. Collection group identified as either of natural or presumed mixed natural and hatchery origin (Mixed). Water temperature at each collection site reported.

Site / Date / Group		mg / dL
WCT 31May Natural		2.5 ± 0.5
N = 5 15.6°C		
WCT 12June Mixed		2.8 ± 0.4
N = 12 16.0°C		
Estuary 22June Mixed		
17°C (surface)		
Severe enteritis,	**n = 5**	0.8 ± 0.2
C.shasta undetected,	**n = 5**	2.5 ± 0.7
Pecwan 22June Mixed		
N = 9 22.0° C		1.0 ± 0.2
TRH 14 days of exposure		
N = 12 MDT 23.7 °C		3.5 ± 0.2

Conclusions:

Despite 37 days of water temperatures in excess of 21° C, overall health of juvenile Chinook salmon examined at WCT during June and July 2001 was considered to be quite good. This observation is based on both the low mortality and low incidence of infection data. In contrast, Ceratomyxosis resulted in a high incidence of sick smolts in both the Klamath R. and estuary. It is unclear to what degree Trinity R. fish are affected by Ceratomyxosis. Ceratomyxosis occurred when water temperatures were below 20 °C. Another myxosporean parasite was observed in the kidney of fish from the Trinity R., Klamath R., and estuary. Particularly in the estuary, infections were associated with marked swelling of the kidney due to glomerulonephritis. The effect of such kidney damage on smolt survival is not known. Some fish appeared to recover from this infection. Bacterial or viral infections were not judged to be significant health problems. Elevated water temperature in the Klamath R. and estuary may have resulted in lower muscle lipid content and gill ATPase activities. Ceratomyxosis resulted in elevated blood neutrophil numbers and lysozyme activity. Plasma protein values were also reduced in the *C.shasta* affected fish.

Future monitoring efforts should concentrate on the incidence and severity of infection of *C. shasta,* kidney myxosporean, and external Columnaris in the estuary. This site will allow for comparison of fish from both Rivers. Marked fish will need to be carefully surveyed to help answer whether Ceratomyxosis effects Trinity R. fish. The large number of sampling sites for smolts and water temperature in the Trinity and Klamath Rivers should make it possible to develop correlations (e.g. Spearman test) of water temperature with disease.

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Appendix 1. data fields:

Site
Date
Collector
water temp/DO
total catch
total   species X   (chinook, steelhead, coho, non-salmonid)
      moribund X
       % moribund X

<u>individual data</u>
CHK ad-clip
head tag number
moribund
normal appearance/behavior
Skin   0=normal, 1 = >30% scale loss, 2= petechial hemorrhage, 3=lamprey bite, Fc erosion

Gill   0 = normal, 1=pale, 2= Fc erosion, 3=copepod, 4= cysts
imprint slide number
long filamentous GNR
histo tube #
histo result

Kidney 0 = normal, 1 = mild swelling, 2 = necrotic/ discolored / swollen 2x normal size
spleen imprint slide number
kidney or spleen TYE culture tube #
FC
kidney or spleen BHIA tube #
isolate
gram stain result
histo tube#
histo result
Metacercaria bag #
metacercaria count   0 = 0 – 5, 1 = 6 – 20, 2> 20
Rs result

Intestine 0 = normal, 1 = pale yellow color fluid / swollen, 2 = hemorrhagic
histo tube #
histo result (CS rating)

Eye   0= normal, 1 = exophthalmia, 2 = hemorrhagic, 3 = cataract/opaque
Vfat 0,1,2,3
FL
Wt
KFL

Aug. 1, 1996

Ceratomyxa Test Results

SHASTA RIVER

Shasta strain rainbows were held in the Shasta River from 5-2 to 6-25-96 (54 days) to test for the presence of Ceratomyxa. Water temperature varied but was above 60 degrees most of the time.

A few fish were lost to unknown causes. None of the survivors were exhibiting disease symptoms after 54 days. Histological samples from 20 fish were negative for this pathogen.

This river was tested for Ceratomyxa in 1987 by Annelise Carleton (HSU) and found negative.

Based on the above findings, its unlikely the Shasta River contributed to the infection of chinook smolts in the Klamath River in 1995.

CRYSTAL LAKE

Chinook salmon smolts from Iron Gate Hatchery and Shasta rainbows from Crystal Lake Hatchery were placed in Ceratomyxa infected water from 7-20 to 8-9-95. By early Sept., RTS were exhibiting disease symptoms while the KS were not. Half of the KS were re-exposed from 9-14 to 10-3-95. The observation period was terminated for all groups on 11-14-95. Ceratomyxa caused the loss of all RTS but was never detected in any KS.

Yearling steelhead from Iron Gate Hatchery and Shasta rainbows from Crystal Lake Hatchery were placed in Ceratomyxa infected water from 4-18 to 7-12-96 (85 days). Water temperature was 50-55 degrees. Ceratomyxa caused the loss of all RTS within 62 days but was never detected in any SH. Histological samples from 10 SH were negative.

The Iron Gate KS and SH demonstrated natural immunity to this pathogen. However, in 1995, morbid KS smolts were found in the Klamath River after release from the hatchery. Dr. Foott (USFWS) confirmed the cause as Ceratomyxa. Could the same thing be occurring in the SH after their release?

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